THE USE OF DETERGENTS FOR DIRECT MYCOLOGIC EXAMINATION*

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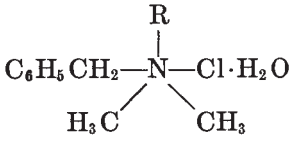
The direct mycologic examination of scales, hairs and nail scrapings is very important for a rapid and correct diagnosis of many skin diseases in a dermatologic department.

An ideal direct examination should fulfill six conditions: 1. good clearing of the material; 2. easy and immediate staining; 3. demonstration of mycelium, spores and bacteria simultaneously; 4. potential implantation of the material used for direct examination into a culture medium; 5. minimum of artefacts; 6. conservation of the slides for demonstration purposes.

MATERIAL AND METHODS

Scales, hairs or nail scrapings were prepared with an aqueous solution of 0.1 per cent Aminol (technical quality, Union Chimique Belge) and 0.2 per cent basic fuchsin in a manner similar to the usual potassium hydroxide mounts, but without any heating.

Aminol is a cationic detergent:



where R is a complex alkyl chain.

The mounts are ready for examination within 2 to 10 minutes. This time depends, of course, on the nature and the thickness of the material.

RESULTS AND DISCUSSION

1. Clearing of the material

Various methods have been utilized for the clearing of scales, hairs and nail scrapings (Table 1). There are, of course, potassium hydroxide mounts and the chloral lactophenol technic of Amann (1). More recently, two anionic detergents, sodium lauryl sulfate and sodium lauryl sulfonate, have been used in 5 per cent and 7.5 per cent aqueous solution for hairs, scales and nail scrapings (2, 3).

However, the surface-active properties of detergents are generally optimal in 0.05 to 0.1 per cent solution, and this is the reason why such low concentrations have been utilized in the procedure here described.

With this method, the clearing of hairs, scales and nail scrapings was very satisfactory. It was not as pronounced as with the chloral lactophenol technic of

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TABLE 1

Results obtained with various substances used as clearing agents

	Potassium Hydroxide	Chloral Lactophenol	Sodium Lauryl Sulfate	Aminol
Clearing	+	++	±	+
Extemporaneous staining	−	−	−	+
Bacteriologic examination	−	−	−	+
Culture medium implantation	−	−	+	±*
Artefacts	+	−	+	−
Demonstration purposes	−	+	+	+

* In the course of this study a nonionic detergent (Belsam III 50 U.C.B.) seems particularly interesting; it possesses all the properties of Aminol but without antifungal effects *in vitro*. It is a mixture of polyglycolic esters from stearic acid. We intend to study its effects in direct mycologic examination and consecutive implantation into culture medium.

Amann (Table 1), but the microscopic examination was as good as with the latter.

2. Staining

Staining methods are generally not easy to perform because of the various manipulations involved (4–14). With the incorporation of the dye into the solution of detergent, clearing and staining require only a few minutes, with no subsequent manipulations.

Staining is uniform in small scales, but with large ones staining is better in their outer area than in the center. Mycelium and spores are red or pink, but always more intensely stained than the scales. The outlines of cells or nuclei (in cases of parakeratotic scales) are sometimes very sharp.

Short hairs coming from tinea capitis, kerion of the scalp and sycosis barbae give pretty pictures. The horny cells are well stained and have sharp outlines. They seem to be dissociated from one another and isolated in the field. The end point of keratolysis by dermatophytes which destroy and break the hair (15–21) can be very well studied with the aid of the surface-active cationic agent Aminol. The red staining of hyphae and spores permits an easy identification of *Microsporon*, *Microides*, *Megasporon* and *Endothrix* types.

Preparations of favus are not so well stained because of the length of the hair and the fact that the keratolytic dissociation is not so complete. Penetration of the detergent takes a long time and is irregular. However, even with alternating stained and unstained areas, the clearing is always sufficiently good. In cases of favus, the hair must be pressed between slide and coverglass because of the thickness and opacity of the follicular end. Mycelium of the favic cup is well stained. Bacterial superinfection is always present in these cases.

Mycelial threads and chains of spores are irregularly stained pink inside the hairs. Around the mycelium, the so-called ribbons of air described by Sabouraud and Rivalier (22–24) appear as thin black bands. Sometimes, a few of these black ribbons move under the microscope along the great axis of the hair, gradually uncovering the mycelium.

Four basic dyes were tried: basic fuchsin, Nile blue, Bismarck brown and Janus green. All of these four dyes stained mycelium and spores in the presence of detergent, but the best results were obtained with basic fuchsin.

3. Demonstration of mycelium, spores and bacteria simultaneously

In potassium hydroxide mounts, or with the chloral lactophenol technic, bacteria are destroyed. With a 5 per cent aqueous solution of sodium lauryl sulfate or sodium lauryl sulfonate and basic fuchsin, clearing is not so good, the bacteria are not stained and sometimes foam interferes with the interpretation of the results (3).

On the contrary, with Aminol and a basic dye, a direct bacteriologic as well as mycologic examination is possible with the mount.

4. Implantation of material used for direct examination into culture medium

Mandel *et al.* (2) demonstrated that it was possible to obtain with preparations in sodium lauryl sulfate successful cultures in Sabouraud's medium. These authors obtained the same percentage of positive cultures from 48 skin or nail scrapings microscopically positive treated with 5 per cent Duponol C in water and from 48 controls (2).

The study of implantation into Sabouraud's medium of material used in the Aminol-basic fuchsin preparations has only begun, but although the results are still not uniform, hairs give positive results more often than do scales. Aminol appears to have antifungal properties, depending upon the concentration of the surface-active agent and the contact time.

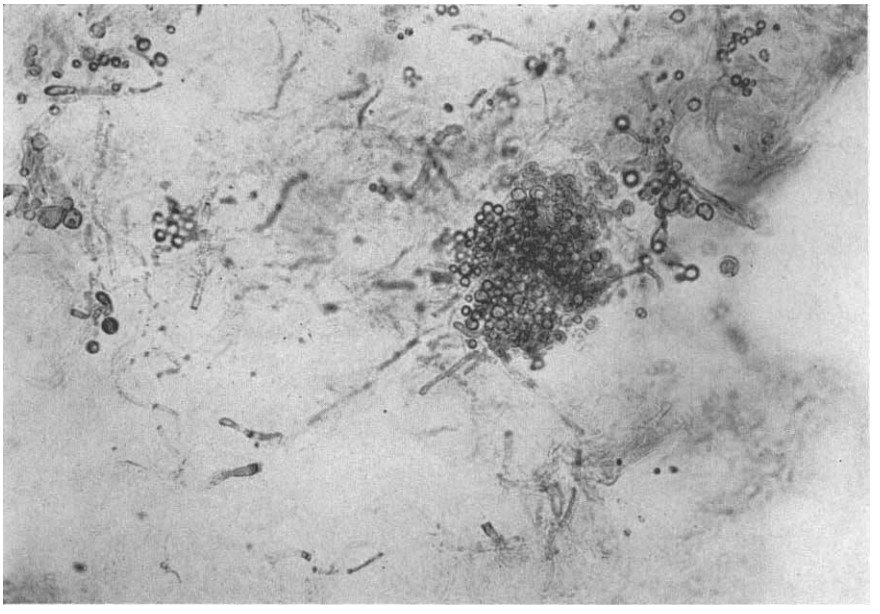

FIG. 1. Aminol basic-fuchsin. Clusters of fungus spores and mycelium in a scale from tinea versicolor.

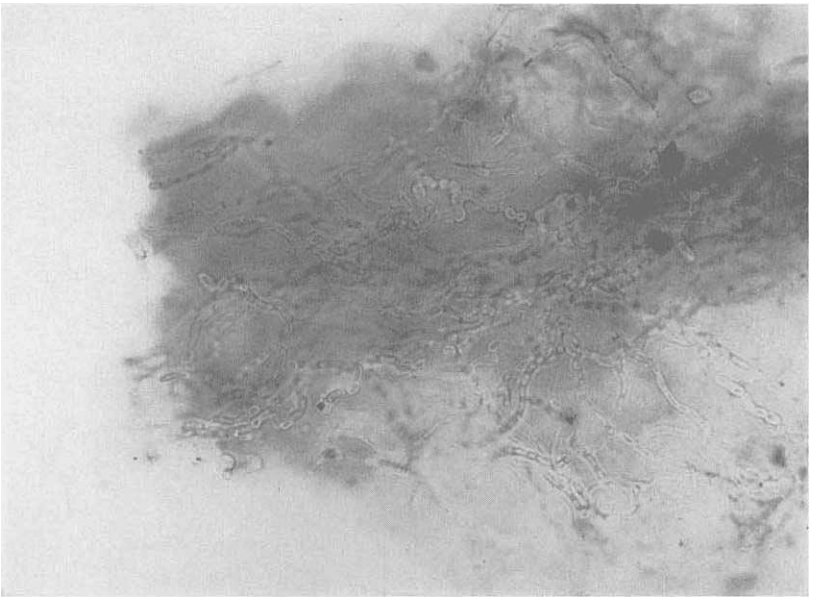

FIG. 2. Aminol basic-fuchsin. Mycelia in scale from dermatomycosis of glabrous skin

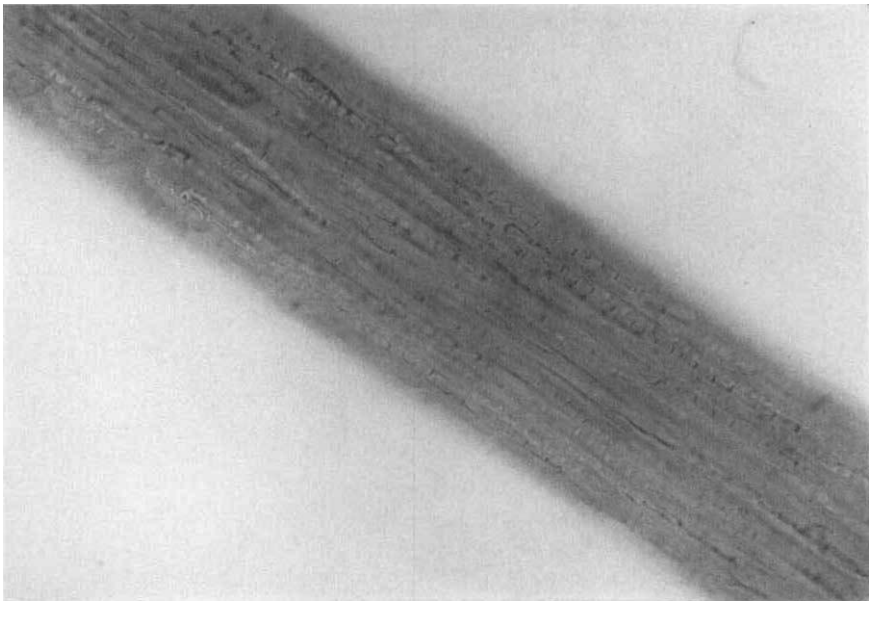

FIG. 3. Aminol basic-fuchsin. Septate mycelium in hair from tinea favosa

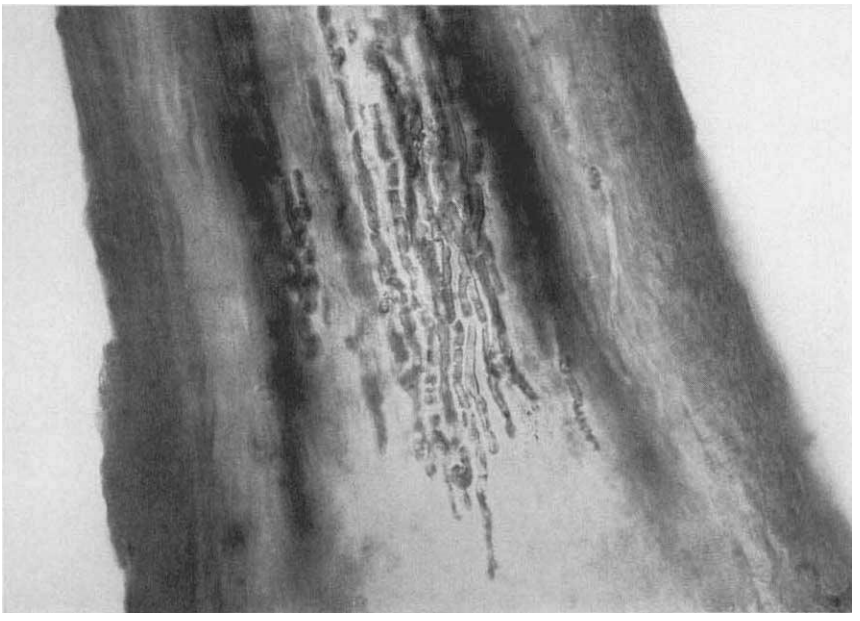

FIG. 4. Aminol basic-fuchsin. Zone of Adamson in tinea favosa

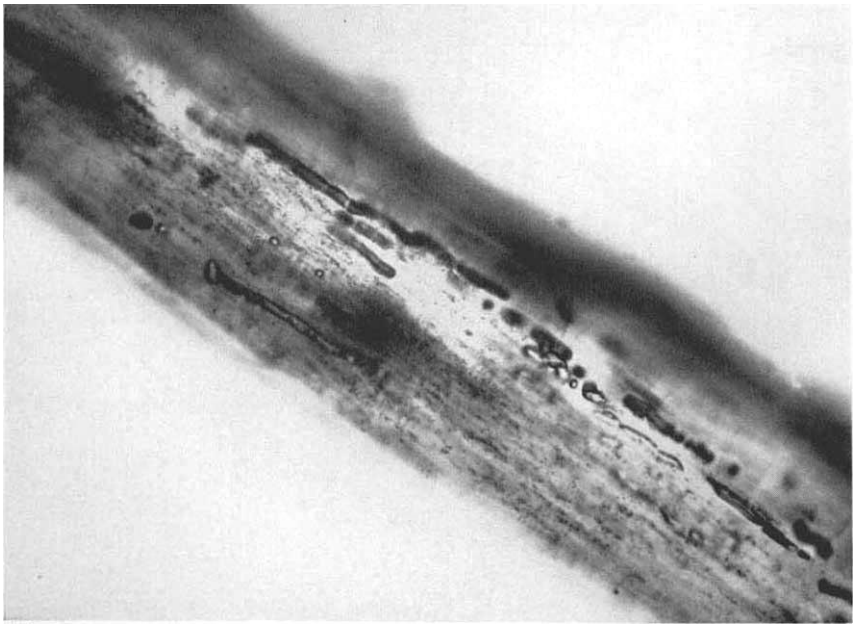

FIG. 5. Aminol basic-fuchsin. Longitudinal air spaces in hair infected with *T. schoenleini*

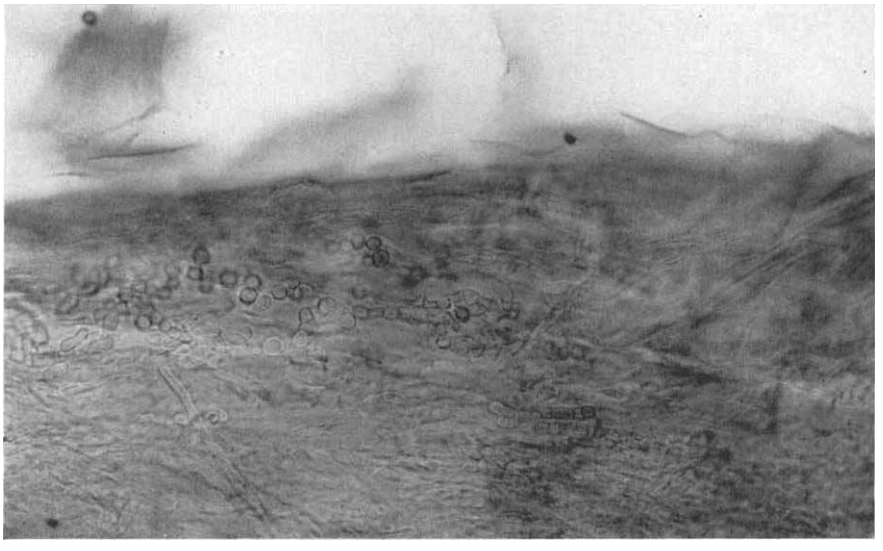

FIG. 6. Aminol basic-fuchsin. Mycelium and spores from sycosis barbae

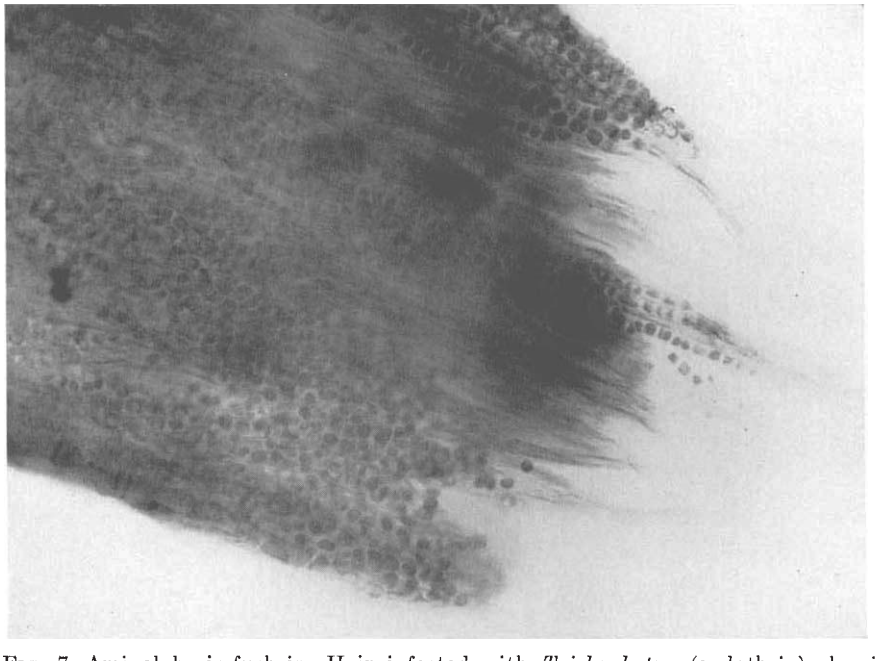

FIG. 7. Aminol basic-fuchsin. Hair infected with *Trichophyton* (endothrix) showing spores inside the hair.

Various modifications of procedure, such as washings, inoculation into fluid medium, and variation of the concentration of detergent and of contact time are now under study.

Material from culture can be stained by the Aminol-basic fuchsin method, but with no advantages over the cotton blue-lactophenol procedure.

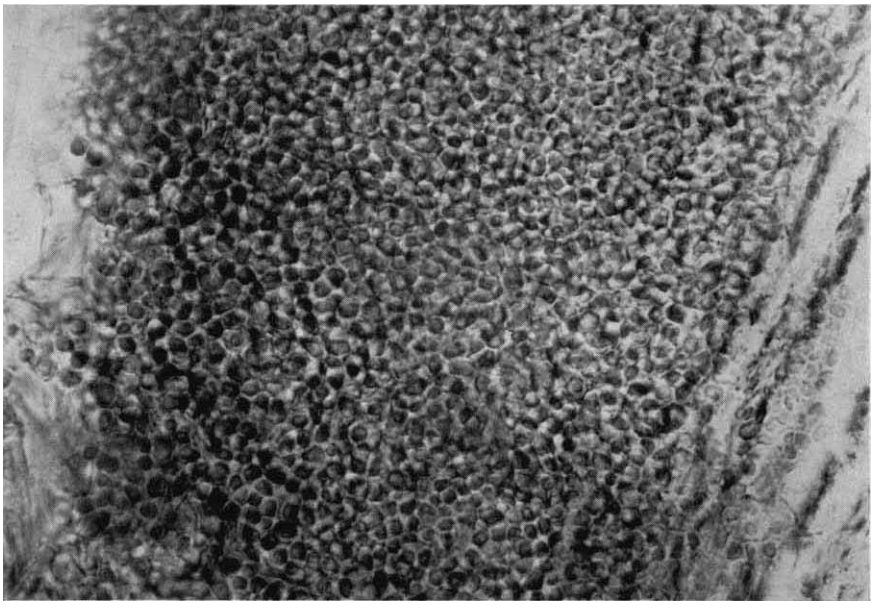

FIG. 8. Aminol basic-fuchsin. Clusters of spores inside the hair of a *Trichophyton* infection.

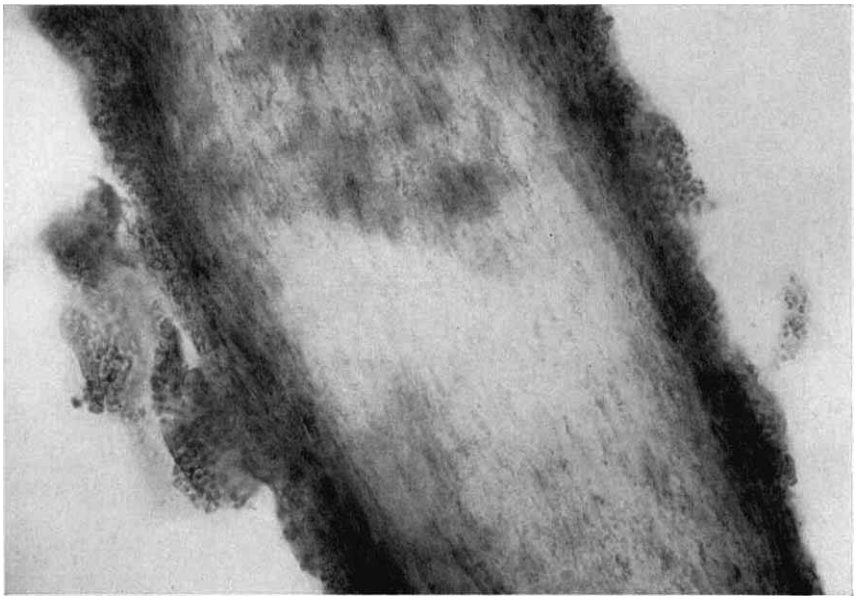

FIG. 9. Aminol basic-fuchsin. Hair infected with *Microsporum* showing small spores around the hair.

5. Artefacts

In the Aminol-basic fuchsin mounts, artefacts are rare. The only ones encountered in direct mycologic examination are bubbles of detergent attached to hairs or scales. These bubbles are iridescent and very different from spores (25).

6. Conservation of scales and hairs for demonstration purposes

It was easy to pick up a scale or a hair and make a permanent balsam mount after rinsing with toluol. Staining and clearing are better in these permanent mounts (Figs. 1–9).

SUMMARY AND CONCLUSIONS

An aqueous solution of 0.1 per cent cationic surface-active agent Aminol (technical quality, Union Chimique Belge, Brussels) and 0.2 per cent basic fuchsin clears scales, hairs and nail scrapings and stains mycelium, spores and bacteria with a minimum of artefacts.

Staining and clearing are achieved in a few minutes. The problem of implanting the material used for the microscopic examination into Sabouraud's medium is still under study.

The procedure described is very simple and constitutes an additional aid in diagnosis.

I would like to express my thanks to Miss J. Burette for her technical assistance in this work.

REFERENCES

1. Amann, J.: Neue Beobachtungsmedien. Ztschr. f. wissensch. Mikr., **16:** 38, 1899.
2. Mandel, E. H., Muskatblit, E., Franks, A. G. and Hermann, F.: New clearing method permitting the same specimen to be examined microscopically for pathogenic fungi and directly inoculated into culture mediums. J. Invest. Dermat., **18:** 61–70, 1952.
3. Delmotte, A. and Bernaerts, J. P.: Note préliminaire sur l'éclaircissement des squames cutanées par le lauryl-sulfate ou le lauryl-sulfonate de sodium. Arch. belges dermat., **9:** 98–103, 1953.
4. Adamson, H. G.: Observation on the parasite of ringworm. Brit. J. Dermat., **7:** 201–237, 1895.
5. Swartz, J. and Conant, N.: Direct microscopic examination of the skin. Arch. Dermat. & Syph., **33:** 297–305, 1936.
6. Swartz, J. and Rockwood: Elements of Medical Mycology, pp. 13–18. New York, Grune and Stratton, 1949.
7. Swartz, J.: Contrast stain for fungi in scales, nails and hair. Hautarzt, **4:** 61–63, 1953.
8. Kligman, A. and Mescon, H.: Periodic acid Schiff stain for demonstration of fungi in animal tissue. J. Bact., **60:** 415–421, 1950.
9. Sharwill, D.: Periodic acid-Schiff stain in diagnosis of dermatomycoses. Brit. J. Dermat., **64:** 329–333, 1952.
10. Lewis, G. M. and Hopper, M. E.: An Introduction to Medical Mycology. Chicago, The Year Book Publishers, Inc., 3rd ed., 1948.
11. Reiss, F.: Mycologic technique, in Simons. "Medical Mycology." Elsevier, 1954.
12. Cohen, M. M.: A simple procedure for staining tinea versicolor (M. furfur) with fountain pen ink. J. Invest. Dermat., **22:** 9, 1954.

13. Chermsirivathana, S.: Rapid method of staining for fungus and monilial infection. J. Invest. Dermat., **19:** 7, 1952.
14. Taschdjian, C. L.: Fountain pen ink as an aid in mycologic technic. J. Invest. Dermat., **24:** 77, 1955.
15. Roberts, L.: The physiology of the Trichophytons. J. Path. & Bact., **3:** 300-309, 1894.
16. Toma, A.: Sur l'infection des cheveux in vitro. Ann. de dermat. et syph., **10:** 641, 1929.
17. Tate, P.: On the enzymes of certain dermatophytes or ringworm fungi. Parasitology, **21:** 31, 1929.
18. Van Breuseghem, R.: Lésions déterminées in vitro par les dermatophytes sur des cheveux isolés. Compt. rend. Soc. de biol., **143:** 1302-1303, 1949.
19. Van Breuseghem, R.: La culture des dermatophytes in vitro sur des cheveux isolés. Ann. de parasitol., **24:** 559-573, 1949.
20. Van Breuseghem, R.: Diagnose et systématique des dermatophytes. Contribution à la connaissance des teignes au Congo belge. Ann. Soc. belge. de med. trop., **30:** 865-986, 1950.
21. Barlow, A. J. E. and Chattaway, F. W.: The attack of chemically modified keratin by certain dermatophytes. J. Invest. Dermat., **24:** 65, 1955.
22. Sabouraud, R.: Les Teignes. Paris, Masson et Cie, 1910.
23. Sabouraud, R.: Maladies cryptogamiques du cuir chevelu. Les Teignes. Nouv. Prat. Dermat. Paris, Masson et Cie, II, 99-221, 1936.
24. Rivalier, E.: Les Teignes, in "Affections de la chevelure et du cuir chevelu" (Desaux), p. 463. Paris, Masson et Cie, 1953.
25. Colson, R.: Précis de cosmétologie, Edit. Opéra—Paris. **4:** 122-125, 1954.